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## Genetic Mutations in African Patients with Atrial Fibrillation: Rationale and Design of the Study of Genetics of Atrial Fibrillation in an African Population (SIGNAL)

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### Abstract

**Background**—There is an urgent need to understand genetic associations with atrial fibrillation in ethnically diverse populations. There are no such data from sub-Saharan Africa, despite the fact that atrial fibrillation is one of the fastest-growing diseases. Moreover, patients with valvular heart disease are under-represented in studies of the genetics of atrial fibrillation.

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**Methods**—We designed a case-control study of patients with and without a history of atrial fibrillation in Kenya. Cases with atrial fibrillation included those with and without valvular heart disease. Patients underwent clinical phenotyping and will have laboratory analysis and genetic testing of >240 candidate genes associated with cardiovascular diseases. A 12-month follow-up assessment will determine the groups' morbidity and mortality. The primary analyses will describe genetic and phenotypic associations with atrial fibrillation.

**Results**—We recruited 298 participants: 72 (24%) with non-valvular atrial fibrillation, 78 (26%) with valvular atrial fibrillation and 148 (50%) controls without atrial fibrillation. The mean age of cases and controls were 53 and 48 years, respectively. Most (69%) participants were female. Controls more often had hypertension (45%) than those with valvular atrial fibrillation (27%). Diabetes and current tobacco smoking were uncommon. A history of stroke was present in 25% of cases and in 5% of controls.

**Conclusion**—This is the first study determining genetic associations in valvular and non-valvular atrial fibrillation in sub-Saharan Africa with a control population. The results advance knowledge about atrial fibrillation and will enhance international efforts to decrease atrial fibrillation-related morbidity.

### Keywords

atrial fibrillation; genetics; case-control study; sub-Saharan Africa

## Introduction

Atrial fibrillation (AF) is the commonest sustained arrhythmia worldwide (1). AF has major public health implications due to its high prevalence, considerable health care costs and morbidity (1). There are almost 11 million people in the United States and Europe with AF and the economic costs reached \$6.7 billion per year in 2005 in the United States (2,3). There is lower prevalence of AF among people of African descent compared to European ancestry in numerous observational studies in high-income countries (4–7). These observed differences in epidemiology may be related to a genetic predisposition that contributes to the risk of AF (2,7–9).

Nonetheless, AF is increasingly common in sub-Saharan Africa. Between 1990 and 2010, there was a 16% increase in age-standardized disability adjusted life years for AF in SSA (10). Rates for most other cardiovascular diseases decreased over the same time period. Moreover, there was a significant decrease in burden of rheumatic heart disease (10), suggesting that over time factors other than rheumatic heart disease will be increasingly responsible for the burden of AF in sub-Saharan Africa. The causes and natural history of AF differ according to region of the world (11,12), yet, genetic associations with AF in sub-Saharan Africa have not been extensively explored.

Until recently, our understanding of the clinical characteristics of AF has also largely been based on data from patients in North American and Europe (13,14). The Randomized Evaluation of Long-Term Anticoagulation Therapy (RELY) AF registry is one of the first multinational registries of AF which includes ten countries in sub-Saharan Africa (15). In

RELY-AF, patients with AF from Africa were seven years younger and more likely to have rheumatic heart disease and heart failure than the overall cohort. While rheumatic heart disease is thought to contribute to risk of AF primarily via hemodynamic and structural changes within the atria, few studies describe genetic associations with AF in patients with valvular heart disease (16,17).

Rheumatic valvular disease is common in sub-Saharan Africa (18), however, other factors related to AF are increasingly common. In Kenyan urban middle to high-income communities, hypertension and diabetes mellitus, and not rheumatic heart disease, are the most common comorbidities in patients with AF (19). Experience from rural and peri-urban settings have not been reported and no study from East Africa has specifically addressed genetic associations with AF. Knowledge of genetic associations with AF in sub-Saharan Africa may identify mechanistic pathways for AF in patients of African descent and may identify molecular targets for screening or treatment.

## Rationale

We identified gaps in the literature related to the under-representation of rural and semi-urban populations from sub-Saharan Africa, the dearth of genetic studies of AF that include participants from Africa, and the lingering uncertainty regarding the extent to which valvular AF related to rheumatic heart disease has genetic determinants. AF has established genetic associations described mostly in populations of European ancestry. This, along with the fact that individuals of African descent have a lower prevalence of AF, suggests that genetic variations contribute to the observed disparities.

The objective of the Study of Genetics of Atrial Fibrillation in an African Population (SIGNAL) is to broaden our understanding of AF by focusing on the following aims in a predominantly rural/semi-urban population in western Kenya:

- Aim 1. To characterize the population of patients with valvular and non-valvular AF clinically and phenotypically
- Aim 2. To describe the presence of mutations in candidate genes in patients with non-valvular AF and compare it against non-AF control subjects
- Aim 3. To describe the presence of mutations in candidate genes in patients with non-valvular AF and compare it against valvular AF
- Aim 4. To assess the presence of mutations in candidate genes in patients with AF (valvular and non-valvular combined) and compare it against non-AF control subjects if there is no difference between those with non-valvular and valvular AF
- Aim 5. To contribute to and participate in additional discovery and validation studies of genetic associations with AF requiring larger sample sizes than are available in this study.

The study aims related to genetic analyses (Aim 2, 3 and 4) are depicted in Figure 1. We hypothesize that genetic mutations, particularly those related to ion channel proteins (20), will be more common in the non-valvular AF group than in the non-AF control group (Aim

2). If proven, this would indicate a genetic association for non-valvular AF in a Kenyan population. We further hypothesize that genetic mutations associated with AF will be more common in the non-valvular AF group compared to the valvular AF group (Aim 3). If this hypothesis was proven, we would interpret this to indicate that the relationship between valvular disease and AF does not necessitate a genetic predisposition. In the event that the hypothesis for Aim 3 is not correct (i.e., there is no difference in genetic mutations between valvular and non-valvular AF), then we would combine these two AF groups and compare them to the non-AF control group (Aim 4) yielding a more precise estimate of the association between genetic mutations and AF. This study will establish the frequency of a number of genetic variants in AF patients in Kenya and will describe their clinical characteristics and overall morbidity. By elucidating any genetic associations, we hope that this study advances the knowledge of the field and provides complimentary information alongside large, international efforts to reduce morbidity related to AF..

## Methods

### Study setting

Participants were recruited from the Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya within the Academic Model Providing Access to Healthcare (AMPATH) program (Figure 2). The AMPATH program is a collaboration between MTRH, Moi University School of Medicine, and a consortium of North American universities that focuses on improving the health of the people of Western Kenya as previously described (21). MTRH is an approximately 750-bed university-affiliated hospital that serves a catchment area of over 20 million people. MTRH provides health care to a broad mix of urban middle class, urban poor and the rural population in Western Kenya (22). Moi University is home to an NHLBI-sponsored Cardiovascular and Pulmonary Disease Center of Excellence in Cardiovascular and Pulmonary Diseases (COE) (23), and is the hub of clinical research in cardiopulmonary diseases in Western Kenya (24).

### Study population and design

The SIGNAL study is a case control study that characterizes patients with and without AF in western Kenya. The study sample is a convenience sample of patients at MTRH. The participants include two groups of AF patients, those with valvular AF (mostly rheumatic) and those with non-valvular AF. All patients being evaluated at cardiology or other medical clinics, the anticoagulation clinic or the cardiac noninvasive diagnostic unit, with a diagnosis of AF, were eligible for participation as AF cases. In addition, there are an equal number of controls without AF (Figure 3). Control patients were recruited from the same clinics and were free of a history of AF. All participants underwent an initial evaluation at baseline and will undergo a 1-year follow-up assessment.

### Inclusion and exclusion criteria

All patients aged ≥ 18 years with a diagnosis of AF were eligible for enrollment as cases. AF had to be recorded on at least one 12-lead ECG. Both prevalent and incident cases of AF were included and no distinction was made according to duration of AF episodes. The diagnosis of non-valvular AF was based on ruling out significant valvular heart disease

through a combination of history, echocardiogram, ECG and clinical findings. Patients with known genetic syndromes, congenital heart defect and other severe illnesses precluding ECG or echocardiogram were excluded (Figure 3).

### Data collection

Data were collected by structured questionnaires, physical examination, venous blood sample analysis, echocardiogram and ECG (Table 1). Research assistants collected data using a structured questionnaire both in Kiswahili and English (Supplementary Material, Appendix A). Each interview lasted approximately 30 minutes followed by physical and clinical measurements. The research participants were expected to complete all the components of the research examination on the same day when possible.

Physical examination included measurement of height, weight, waist and hip circumferences, blood pressure, heart rate, and cardiovascular examination. Measurements were taken on individuals in light clothing without shoes. A digital scale was used to record the weights of the subjects to the nearest kilogram. Height was measured using a stadiometer to the nearest 1cm. Both waist and hip circumference were measured to the nearest cm using a plastic measuring tape. Body mass index (BMI) was calculated using the measured weight and height ( $\text{kg/m}^2$ ). Prior to blood pressure measurements, participants were asked to sit quietly for 5 minutes with arm supported on a table placed at the level of the heart. Bilateral blood pressure was taken twice with an automatic sphygmomanometer (Omron Hem 712c, Omron Healthcare, Kyoto, Japan), with a two-minute interval between measurements.

Blood samples were collected in BD Vacutainer CAT (5ml) and K<sub>2</sub>-EDTA vacutainers (10ml  $\times$  2 and 5ml). Samples were typically processed within 4 hours of collection. 5ml BD Vacutainer CAT was centrifuged at 3000rpm for 4 min and serum was collected for laboratory analysis. These analyses included basic chemistry, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, B-type natriuretic peptide, thyroid stimulating hormone, and C-reactive protein. 5ml K<sub>2</sub>-EDTA vacutainer destined for glycated hemoglobin was not centrifuged but stored at  $-4^\circ\text{C}$  for subsequent analysis. For genetic analysis, the two–10ml K<sub>2</sub> EDTA tubes were centrifuged, buffy coat separated and stored at  $-80^\circ\text{C}$  prior to shipping one sample for each participant to the Indiana University Biobank in Indianapolis, Indiana, USA for genetic analysis. Two peripheral blood mononuclear cell (PBMC) pellets were stored for each participant; one in Indianapolis, Indiana and one in Eldoret, Kenya.

### Electrocardiogram and Echocardiogram

12-lead resting ECGs were performed using a Pagewriter TC 30 ECG machine (Philips Healthcare, Andover, Massachusetts, USA) and stored as portable data files for0020analysis. All echocardiograms were performed and digitally acquired using a VividQ (General Electric Medical Systems, Hortan, Norway) by an experienced sonographer. A number of imaging windows and views were used in accordance with guidelines of the American Society of Echocardiography (25). Two-dimensional loops, M-mode, Doppler and speckle tracking imaging were used to assess cardiac structure and function. Images were

stored in DICOM format and transported to a core echocardiography laboratory at Duke University Medical Center for analysis.

All image analysis and quantification will be performed by cardiologists with expertise in echocardiography who are blinded to the clinical information. A reading protocol will be followed that includes an assessment of the structure, function and hemodynamic performance of all cardiac chambers. Standard guidelines will guide assessment of dimensions (25), diastolic function (26), rheumatic heart disease (27), valvular function (28,29) and hemodynamics (30) of the left and right heart (31).

### Genetic Analysis

We will use a custom HaloPlex Enrichment kit (Agilent) to target the coding sequence and exon/intron boundaries of >240 genes with strong scientific evidence for a causative role in the development of cardiovascular disease in people of European ancestry, 53 of which are linked to cardiac arrhythmias (Supplementary Material, Appendix B). The genetic associations in familial AF are described in the Online Mendelian Inheritance in Man database (<http://www.omim.org/entry/608583>). The frequencies of mutations in the 53 arrhythmia-related genes of interest in this study is not known, however, a recent analysis of 14 AF-associated genes (KCNQ1, KCNH2, SCN5A, KCNA5, KCND3, KCNE1, KCNE2, KCNE5, KCNJ2, SCN1B, SCN2B, SCN3B, NPPA, and GJA5) in 192 Danish Caucasian patients with onset of lone atrial fibrillation before 40 years of age revealed rare variants (minor allele frequency [MAF] of <1%) in 29/192 (15%) subjects (32). SCN5A covered 28% of all variants identified in that cohort, followed by KCNQ1 (14%), KCNA5 (14%), SCN3B (10%), SCN2B (7%), KCNE1 (7%), KCNE2 (7%), while the other genes each had one variant identified.

Genomic DNA will undergo library preparation using the custom HaloPlex Target Enrichment kit (Agilent), which will fragment the DNA to a suitable range (300–600bp) and will apply specific adapter sequences on both ends. The adapters are complementary to platform-specific polymerase chain reaction (PCR) and sequencing primers. Each sample will undergo molecular tagging with unique sequence-based codes (barcoding) to allow sample pooling in the same run. Library preparation will undergo quality control (QC) using a TapeStation, which will be employed before library preparation and quantitative real-time PCR that will be used after library preparation. These steps will provide the necessary metrics to assess the efficient fragmentation within the desired size range and the successful adapters/barcoding addition to each sample's DNA fragment. Library will be used to generate sequencing data using a next generation sequencing (NGS) Illumina MiSeq desktop sequencer, which will acquire sequencing data point and generate a .bam and a .fastq files for sequence reads. The custom gene panel was designed to achieve an average depth of coverage of 200×, which represents a clinical test level of quality to detect constitutive genomic variants. Variant calls will be generated using the Burrows-Wheeler Aligner (bwa) followed by GATK analysis, which will generate a variant call format (.vcf) file to be used for final interpretation. Sanger sequencing will be used to provide data for bases with insufficient coverage using the NGS approach and for variant confirmation. The sequencing analysis will be performed in a clinical laboratory at Indiana University with experience in



these techniques, which assures high quality control due to the implementation of specific standard operating procedures for reproducibility and the use of positive and negative controls to assess the quality of the PCR amplification and sequencing data.

We will sequence all nucleotides in the coding sequence and immediate intronic region at the exon/intron boundaries of 246 genes. We are not using genotyping methods that target specific single nucleotide polymorphisms (SNPs). We are capturing the coding sequence and the whole exon and exon/splice site boundaries of each gene and will, therefore, capture all variants present in the exons that are successfully sequenced.

### **Follow-up**

A twelve-month follow up assessment will capture the incidence of cardiac and non-cardiac medical events, mortality, medication usage, hospitalizations, and changes in life habits from baseline (Table 1). In the case of out of hospital deaths, information will be collected by interviewing physicians or next of kin.

### **Sample size considerations**

The sample size consideration is driven by Aim 2. With 70 cases of non-valvular AF and 140 controls and assuming the mutation rate in the control group is close to 0%, we will have 82% power to detect a difference in the mutation rate between cases and controls as small as 8% with a one-sided type I error rate controlled at 0.05.

### **Ethical considerations**

The study protocol and data collection forms were approved by the Institutional Research and Ethics Committee of Moi University School of Medicine (FAN #: IREC 1028) and the Institutional Review Boards of Indiana and Duke University. All participants provided informed consent with the knowledge that the planned genetic studies include analysis of multiple genes associated with cardiovascular diseases. The results of research testing were not placed in the medical record. Study participants received information on relevant medical tests at their request. In addition, any findings that were felt to warrant immediate medical attention were reported to the participant and their physician. This study was funded by the Indiana University Health-Indiana University School of Medicine Strategic Research Initiative. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.

## **Results**

### **Status of the study and study participants**

Enrollment occurred between October 2013 and July 2014. Figure 4 shows the flow of patients recruited into the study. Of 428 patients screened, 298 subjects (70% enrollment rate) were enrolled. Table 2 shows the baseline characteristics of our study population. We enrolled 72 patients with non-valvular AF, 78 patients with valvular AF and 148 controls yielding 298 participants. Among the 298, 285 subjects (95%) completed all of the study related investigations. In a few instances, the study investigations could not be completed during the same day as enrollment. The majority of our participants (206 of 298, 69%) were

female. At enrollment, the average ages of all cases and all controls were 53 and 48 years, respectively. Non-valvular AF cases were, on average, 30 years older than valvular AF cases (68 vs. 38 years,  $p<0.001$ ). Current tobacco smoking was uncommon in this population, however, 32% of non-valvular AF patients reported former smoking. A history of hypertension was reported in 45% of controls and 46% of cases (67% in non-valvular AF and 27% in valvular AF,  $p<0.001$ ). Diabetes was uncommon in AF cases. A history of stroke was reported in 5% of controls and 24% of cases. There was no statistically significant difference in stroke history between non-valvular (25%) and valvular AF (23%) cases. Planned analyses include a detailed description of the phenotypic and genetic associations with both forms of atrial fibrillation.

## Discussion

Baseline data of this study indicate that patients with AF in Kenya are younger as compared to Western counterparts, by approximately 10 years (12,15). The age distribution of valvular AF patients being younger compared to non-valvular AF patients follows worldwide patterns (12). Most AF patients in this study were women who also accounted for the largest proportion of cases with valvular AF. This in contrast to the male predominance in Western countries and may be related to healthcare seeking behaviors (33). Self-reported time since AF diagnosis was significantly longer for patients with valvular compared to non-valvular AF. Hypertension is more common in non-valvular AF cases compared to valvular AF and diabetes is uncommon in both groups. These findings may be related to the age difference in these groups and will be explored upon completion of the study. A history of stroke, on the other hand, was present in approximately 25% of patients with AF which stands in stark contrast to estimates for patients in this age range from developed countries (12).

In addition to traditional cardiovascular risk factors, a genetic predisposition has been shown to contribute to risk of AF (9). The first large genome wide association studies of associations with AF were in participants from Iceland, the United States, Germany and the Netherlands all of which were of European ancestry (34–37). These studies identified associations between AF and the 4q25, 1q21 and 16q22 loci. Multiple susceptibility signals have since been found at these and other loci in people of European and Japanese ancestry such that, to date, genome wide association studies have identified twelve common SNPs related to the risk of AF (34–39). People of European ancestry harbor only a fraction of human genetic variation and people of African descent have been under-represented in the studies of genetic associations with AF (40). Studies including African-Americans show that ancestry is related to genetic risk of AF. Among African-Americans, for every 10% increase in measure of European ancestry, there is a 17% higher risk of incident AF (7). Studies of genetic associations with AF that have included people of African descent have suffered from small numbers of African-American individuals and findings should be interpreted with caution (41,42). There, however, are a number of multi-ethnic studies and registries with well analyzed subgroups that could also contribute to genetic associations with AF (43–49) as outlined in a recent review (50). This is the first study of genetic associations with AF in an exclusively East African population. One strength of this study relates to geography and the genetic heterogeneity in this part of the world which, some suggest, is the most genetically diverse on the planet (51–53). The study population consists largely, but not



exclusively, of individuals of Nilo-Saharan ancestry. Individuals in the catchment area of this study do not generally move to other areas barring major circumstances. However, as Eldoret is a cosmopolitan town there are individuals from Bantu, south Indian and other ancestries represented in this study as well. We believe in-migration to be less common than in the Americas. We therefore have an opportunity to identify genetic associations with AF not present in other regions. We have also included patients with valvular AF and are in a unique position to identify genetic and clinical risk factors in this group since patients with valvular AF are usually excluded from clinical studies of AF. Our genetic analysis approach includes >240 genes and offers the opportunity to identify numerous genetic variants that may relate to AF. We also highlight the relative rarity of valvular AF outside of an environment similar to western Kenya as a unique strength of this study.

There are also a number of limitations worth noting. Our sample size is relatively small including 78 patients with valvular AF, 72 with non-valvular AF and 148 controls. Our goal sample size was based on a convenience estimate of the estimated number of non-valvular AF patients in our setting. Non-valvular AF is much less common compared to valvular AF in our setting and we wanted to include relatively equal numbers of patients with both forms of AF. While a larger sample size may yield more powerful results, this is not possible in our setting and studies with smaller sample sizes from the US exploring genetic associations with AF in African-Americans have been able to detect important findings (42). We have also included collaboration and pooling data with other cohorts as one of our specific aims a priori. Our follow-up assessment may be limited by loss to follow-up. To address this limitation, we have focused our follow-up assessment to endpoints that are relatively simple to recognize and report. The lack of awareness of AF and healthcare seeking behavior may result in AF patients being those that are highly symptomatic or having complications. Lastly, we do not differentiate between various types of AF (i.e., paroxysmal, persistent, etc.) in this study and may have missed cases of AF with paroxysmal AF or who develop AF over time.

## Conclusions

Although risk factors for AF are well known, they do not explain all of the risk of AF in populations of African ancestry. Among cardiovascular diseases in sub-Saharan Africa, the burden of AF is increasing the fastest. Numerous contemporary studies have identified genetic variants that relate to higher risk of AF but few have included populations in sub-Saharan Africa. The SIGNAL study will characterize populations with valvular AF, non-valvular AF and a control population with the ultimate goal of identifying both clinical and genetic AF risk factors as a first step towards enhancing our knowledge about AF in sub-Saharan Africa.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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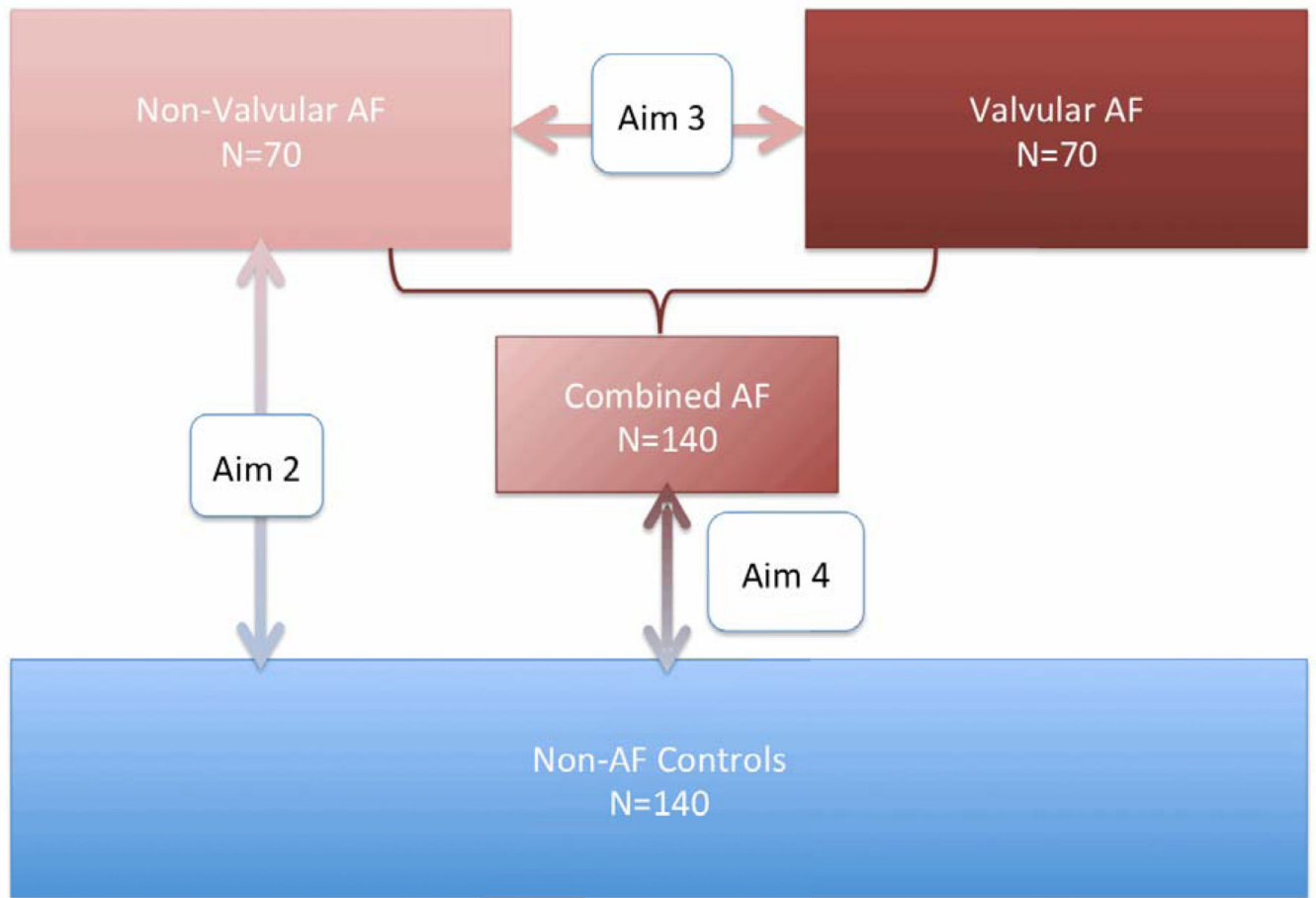
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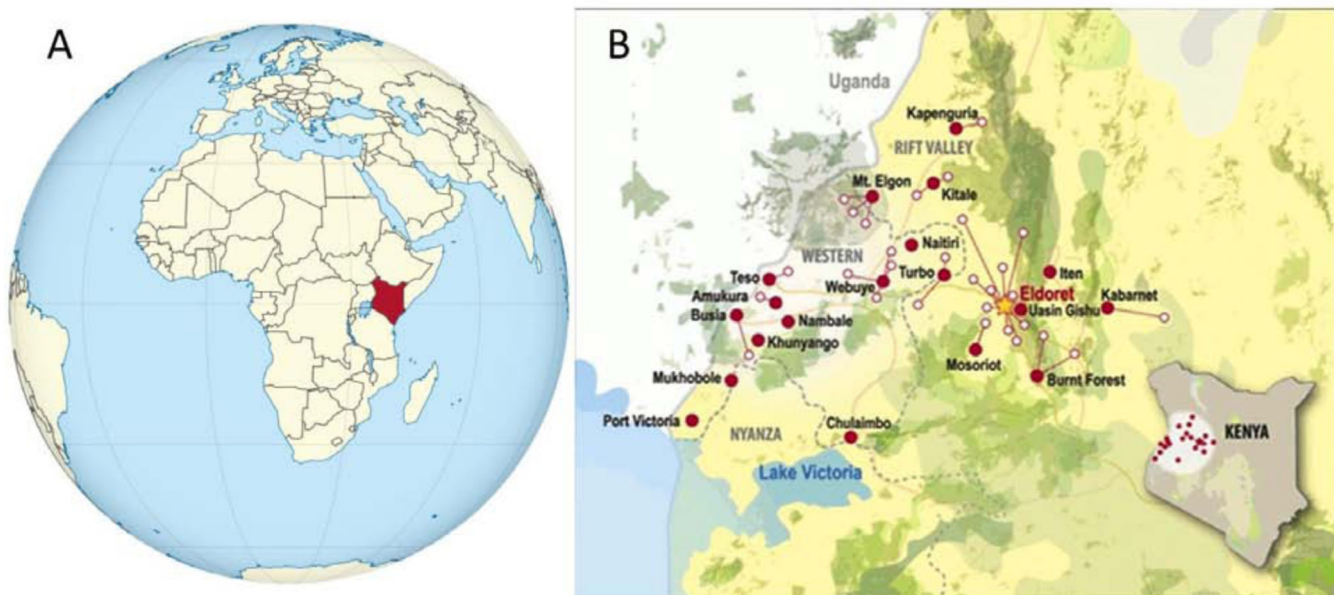
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**Figure 1. Comparator groups for genetic analyses in the Study of Genetics of Atrial Fibrillation in an African Population**

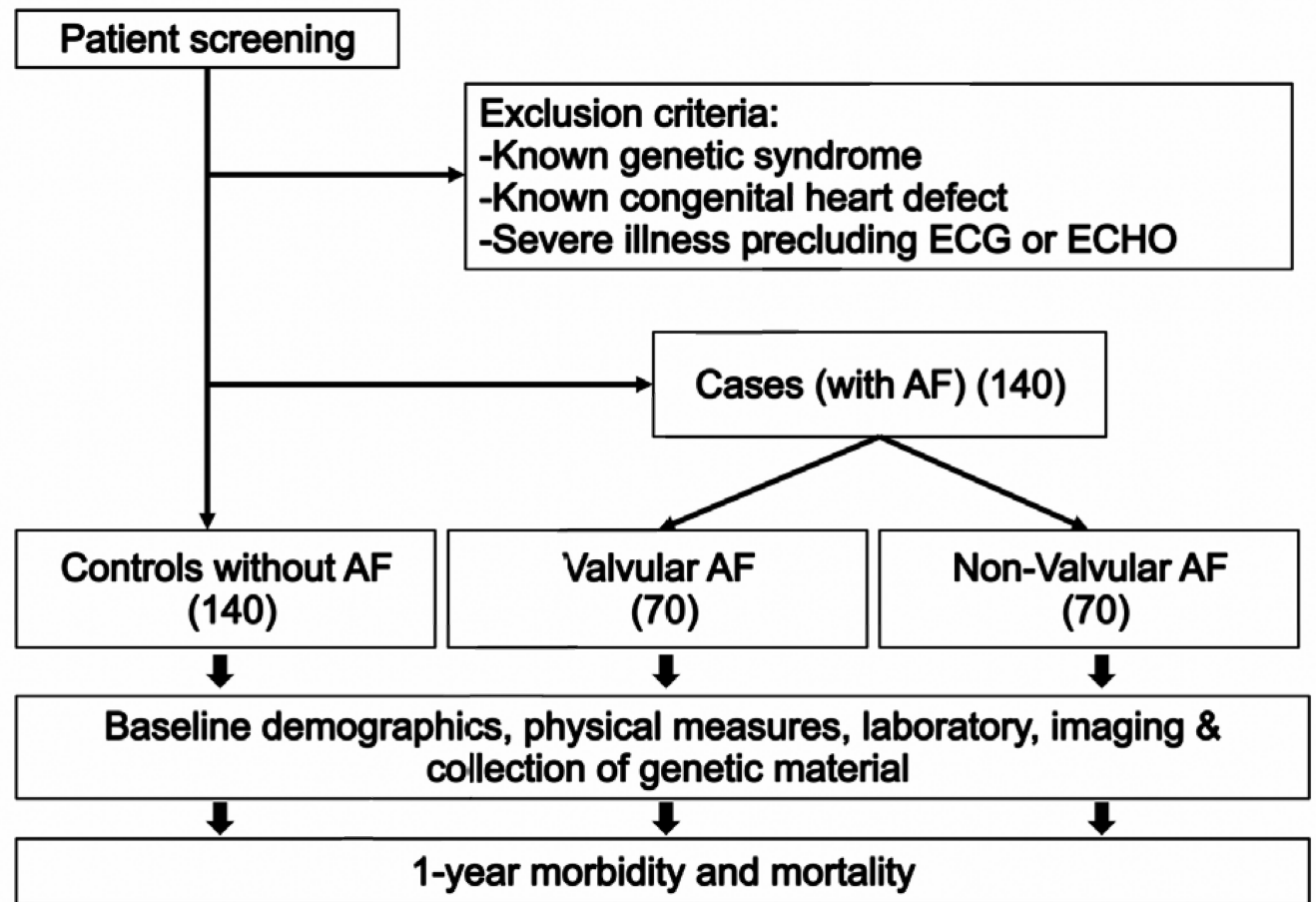
Target enrollment was 140 controls and 70 cases each with valvular and non-valvular atrial fibrillation. See text for full description of study aims.



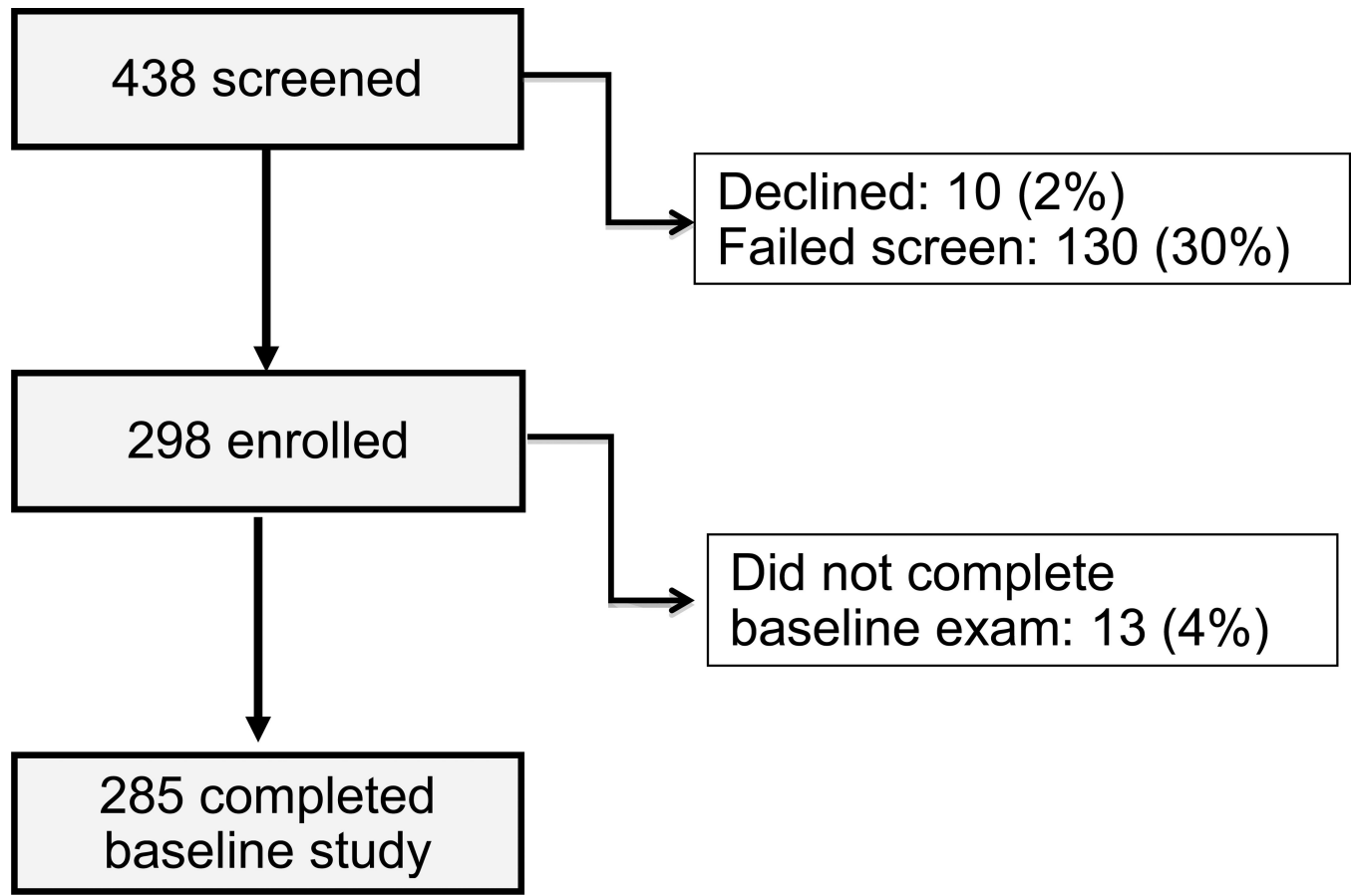


**Figure 2. Map of the study catchment area**

(A) Kenya on the globe and (B) the Academic Model Providing Access to Healthcare (AMPATH) Program Catchment Area in Western Kenya. Panel A figure from Wikimedia Commons ([commons.wikimedia.org](https://commons.wikimedia.org))



**Figure 3. Study enrollment design and exclusion criteria**  
 AF, atrial fibrillation; ECG, electrocardiogram; ECHO, echocardiogram. The SIGNAL study will enroll patients with and without AF to determine genetic associations with AF.



**Figure 4. Flow chart of patients recruited into SIGNAL study**

Of all patients screened, 67% were enrolled and included for analysis. Owing to the need to return for testing, a small number (n=15) did not complete all investigations.

**Table 1**

Components of SIGNAL study by examination

Domain	Variables	Baseline	Follow - up
Questionnaire	Demographics	X	X
	Medical history	X	X
	Social history	X	X
	Medications	X	X
	Physical examination	X	
	Blood pressure	X	
	Anthropometry (height, weight, hip and waist circumference)	X	
	Family history	X	
	Hospitalizations and mortality		X
Blood testing	Na+, K+, Creatinine, BUN, TSH, Lipid		
	Profile, C-reactive protein, HbA1c and BNP	X	
	Genetic analysis	X	
Other tests	Electrocardiogram	X	
	Echocardiogram	X	

**Table 2**

## Baseline characteristics

Variables	Controls (n=148)	Cases nvAF (n=72)	Cases vAF (n=78)	P Value* nvAF vs VAF
Age $\pm$ SD, y	49 $\pm$ 18	68 $\pm$ 13	38 $\pm$ 15	<0.001
Female, n(%)	105 (71)	38 (53)	61 (78)	<0.001
Duration in years $\pm$ SD, y	-	3 $\pm$ 7	7 $\pm$ 7	<0.001
Education level n (%)				<0.001
None	22 (15)	34 (47)	4 (5)	
Primary	53 (36)	24 (33)	37 (47)	
Secondary or higher	73 (49)	14 (19)	37 (47)	
Socioeconomic status, n(%)				0.62
0/5	58 (39)	38 (53)	47 (60)	
1/5	25 (17)	10 (14)	11 (14)	
2/5	39 (26)	10 (14)	8 (10)	
3/5	13 (9)	3 (4)	6 (8)	
4/5	7 (5)	5 (7)	2 (3)	
5/5	6 (4)	6 (8)	4 (5)	
Tobacco history, n(%)				<0.001
Current	4 (3)	4 (6)	1 (1)	
Former	14 (9)	23 (32)	6 (8)	
Never	130 (88)	45 (63)	71 (91)	
Hypertension, n(%)	66 (45)	48 (67)	21 (27)	<0.001
Diabetes, n(%)	22 (15)	6 (8)	1 (1)	0.05
Stroke, n(%)**	5 (3)	18 (25)	18 (23)	0.78

SD- Standard deviation, nvAF- Non-valvular AF, vAF- Valvular AF

\* Wilcoxon rank-sum and Fisher's exact tests used for the comparison of continuous and binary variables, respectively.

\*\*  
n=145